

Amendments to the claims:

This listing of claims replaces all prior versions, and listings, of claims in the application.

Listing of claims:

1 (original): An anti-proBNP(1-108) antibody, characterized in that, firstly, it specifically recognizes the sequence RAPR₇₆S₇₇P (SEQ ID No. 5) of proBNP(1-108) and does not substantially recognize the peptides BNP(1-76) or BNP(77-108) and, secondly, it has the ability to specifically recognize circulating proBNP(1-108) in human serum or plasma samples.

2 (original): The anti-proBNP(1-108) antibody as claimed in claim 1, which specifically recognizes the sequence Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₅ (SEQ ID No. 4) of proBNP(1-108).

3 (original): The anti-proBNP(1-108) antibody as claimed in claim 1, which specifically recognizes the sequence Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₄ (SEQ ID No. 108) of proBNP(1-108).

4 (previously presented): A method for obtaining an anti-proBNP(1-108) antibody as defined in claim 1, in which an animal is immunized with the whole proBNP(1-108) molecule,

and then the antiserum obtained is depleted using the BNP(77-108) peptide and/or the BNP(1-76) peptide.

5 (currently amended): A method for obtaining an anti-proBNP(1-108) antibody as defined in claim 1, in which an animal is immunized with a peptide chosen from

- a peptide of formula



where

a₁ may be H or may represent a function or a chemical group chosen from a thiol, alcohol, aminoxy, primary amine or secondary amine function, an aminocarboxyl group, a biotinyl group and an acetyl group,

X₁ represents a peptide sequence of 0 to 3 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

X₂ represents a peptide sequence of 0 to 7 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

a₂ may represent an OH function, an NH₂ function or an alkoxy group;

- a peptide of formula



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- a peptide of formula



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- a peptide comprising a sequence derived from the sequence

$X-Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{85}-Z$ (II) or from the sequence

$X-Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{84}-Z$ (III) by substitution of one or more among the amino acids Y_{70} , T_{71} , L_{72} , K_{79} , M_{80} , V_{81} , Q_{82} , G_{83} , S_{84} and G_{85} , with it being possible for X to be H or to represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may be an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);;

- the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-G (C13P30: SEQ ID No.16);
- the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S (CN32: SEQ ID No. 109);

and, optionally, the antiserum obtained is depleted using the BNP(77-108) peptide and/or the BNP(1-76) peptide.

6 (currently amended): A method for obtaining a hybridoma that secretes an anti-proBNP(1-108) antibody as defined in claim 1, in which an animal is immunized with a peptide chosen from

- a peptide of formula



where

a_1 may be H or may represent a function or a chemical group chosen from a thiol, alcohol, aminoxy, primary amine or secondary amine function, an aminocarboxyl group, a biotinyl group and an acetyl group,

X_1 represents a peptide sequence of 0 to 3 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

X_2 represents a peptide sequence of 0 to 7 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

a_2 may represent an OH function, an NH_2 function or an alkoxyl group;

- a peptide of formula



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- a peptide of formula



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- a peptide comprising a sequence derived from the sequence

X-Y₇₀TLRAPR₇₆S₇₇PKMVQSGS₈₅-Z (II) or from the sequence

X-Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₄-Z (III) by substitution of one or more among the amino acids Y₇₀, T₇₁, L₇₂, K₇₉, M₈₀, V₈₁, Q₈₂, G₈₃, S₈₄ and G₈₅, with it being possible for X to be H or to represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may be an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-G (C13P30: SEQ ID No.16);

- the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S (CN32: SEQ ID No. 109);

immunoglobulin-secreting lymphocytes are taken from this animal,

and the lymphocytes are fused with myeloma cells so as to obtain at least one immunoglobulin-secreting hybridoma.

7 (previously presented): The method as claimed in claim 5, in which the peptide of formula (II) has the sequence Y₇₀TLRAPR₇₆S₇₇PKMVQSGS₈₅ (SEQ ID No. 4).

8 (currently amended): The method as claimed in ~~either~~ of claim 5, in which the peptide of formula (III) has the sequence Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₄ (SEQ ID No. 108).

9 (previously presented): A hybridoma which can be produced by the method as claimed in claim 6.

10 (original): An anti-proBNP(1-108) monoclonal antibody secreted by a hybridoma as claimed in claim 9.

11 (currently amended): A method of *in vitro* diagnosis of heart failure in a human subject, comprising bringing a biological sample of the human subject into contact with an anti-proBNP (1-108) antibody as defined in claim 1[,] and detecting the proBNP (1-108) in the sample, whereby, if the proBMP (1-108) concentration in the biological sample is higher than that of normal individuals, then the human subject is diagnosed as having heart failure.

12 (currently amended): A method of *in vitro* diagnosis of heart failure in a human subject, comprising:

a) bringing a biological sample of the human subject into contact with an anti-proBNP(1-108) antibody as defined in claim 1,

b) incubating the mixture under conditions that allow the formation of antigen-antibody complexes, and

c) revealing the antigen-antibody complexes formed,

optionally using a labeled detection antibody capable of binding specifically to the proBNP(1-108) present in the primary complex, or using a labeled detection antigen capable of binding to the antibody directed against said proBNP(1-108) present in the primary complex, and

d) correlating the amount of antigen-antibody complexes revealed with the clinical condition of the human subject, wherein, if the amount of antigen-antibody complexes is higher than that of normal individuals, then the human subject is diagnosed as having heart failure.

13 (cancelled):

14 (previously presented): A kit for detecting proBNP(1-108) in a biological sample, containing at least one antibody as defined in claim 1.

15 (currently amended): The kit for detecting proBNP(1-108) in a biological sample, as claimed in claim 14, containing:

(i) in a container, the at least one antibody;

(ii) in another container, at least one peptide chosen from

- a peptide of formula

a_1 -X₁-RAPRSP-X₂-a₂ (SEQ ID No. 5) (I)

where

a₁ may be H or may represent a function or a chemical group chosen from a thiol, alcohol, aminoxy, primary amine or secondary amine function, an aminocarboxyl group, a biotinyl group and an acetyl group,

X₁ represents a peptide sequence of 0 to 3 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

X₂ represents a peptide sequence of 0 to 7 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

a₂ may represent an OH function, an NH₂ function or an alkoxyl group;

- a peptide of formula

X-Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₅-Z (SEQ ID No. 4) (II)

where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- a peptide of formula

X-Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₄-Z (SEQ ID No. 108) (III)

where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

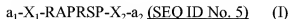
- a peptide comprising a sequence derived from the sequence

X-Y₇₀TLRAPR₇₆S₇₇L₇₈PKMVQGS₈₅-Z (SEQ ID No. 4) (II) or from the sequence

X-Y₇₀TLRAPR₇₆S₇₇L₇₈PKMVQGS₈₄-Z (SEQ ID No. 108) (III) by substitution of one or more among the amino acids Y₇₀, T₇₁, L₇₂, K₇₉, M₈₀, V₈₁, Q₈₂, G₈₃, S₈₄ and G₈₅, with it being possible for X to be H or to represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may be an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108);

- the peptide having the sequence Y₇₀TLRAPR₇₆S₇₇L₇₈PKMVQGS₈₅ (SEQ ID No. 4);
- the peptide having the sequence Y₇₀TLRAPR₇₆S₇₇L₇₈PKMVQGS₈₄ (SEQ ID No. ~~409~~ 108);
- the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-G (C13P30: SEQ ID No.16);
- the peptide having the sequence C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S (CN32: SEQ ID No. ~~408~~ 109).

16 (currently amended): A peptide of formula:



where

a₁ may be H or may represent a function or a chemical group chosen from a thiol, alcohol, aminoxy, primary amine or secondary amine function, an aminocarboxyl group, a biotinyl group or an acetyl group,

X₁ represents a peptide sequence of 0 to 3 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

X₂ represents a peptide sequence of 0 to 7 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

a₂ may represent an OH function, an NH₂ function, or an alkoxy group.

17 (currently amended): A peptide of formula



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108).

18 (original): The peptide as claimed in claim 17, having the sequence
Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₅ (SEQ ID No. 4).

19 (currently amended): A peptide of formula:



where X may be H or may represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may represent an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108).

20 (original): The peptide as claimed in claim 19, having the sequence Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₄ (SEQ ID No. 108).

21 (currently amended): A peptide comprising a sequence derived from the sequence X-Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₅-Z (SEQ ID No. 4) (II) or from the sequence X-Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₄-Z (SEQ ID No. 108) (III) by substitution of one or more among the amino acids Y₇₀, T₇₁, L₇₂, K₇₉, M₈₀, V₈₁, Q₈₂, G₈₃, S₈₄ and G₈₅, with it being possible for X to be H or to represent either an acetyl group, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108), and where Z may be an OH function, or 1 to 3 amino acids not belonging to the sequence of proBNP(1-108).

22 (previously presented): The peptide as claimed in claim 16, having a sequence chosen from the group consisting of the following sequences

SEQ ID No. 16: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-G (peptide C13P30)

SEQ ID No. 109: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S (peptide CN32)

SEQ ID No. 6: C-G-R-A-P-R-S-P

SEQ ID No. 7: Acetyl-C-G-R-A-P-R-S-P

SEQ ID No. 8: C-G-R-A-P-R-S-P-K

SEQ ID No. 9: Acetyl-C-G-R-A-P-R-S-P-K

SEQ ID No. 10: C-G-R-A-P-R-S-P-K-M-V

SEQ ID No. 11: C-G-R-A-P-R-S-P-K-M-V-Q-G-S-G
SEQ ID No. 12: R-A-P-R-S-P-G-C
SEQ ID No. 13: Acetyl-R-A-P-R-S-P-G-C
SEQ ID No. 110: C-Y-T-L-R-A-P-R-S-P-K
SEQ ID No. 111: C-Y-T-L-R-A-P-R-S-P-K-M-V
SEQ ID No. 112: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q
SEQ ID No. 113: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G
SEQ ID No. 19: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-bA
SEQ ID No. 20: C-Y-T-L-R-A-P-R-S-P-K-M-V-Q-A-T-bA
SEQ ID No. 114: Acetyl-C-T-L-R-A-P-R-S-P-K-M-V-Q
SEQ ID No. 115: C-T-L-R-A-P-R-S-P-K-M-V-Q-G
SEQ ID No. 116: C-T-L-R-A-P-R-S-P-K-M-V-Q-G-S
SEQ ID No. 117: C-T-L-R-A-P-R-S-P-K-M-V-Q-G-S-G
SEQ ID No. 118: C-L-R-A-P-R-S-P-K-M-V
SEQ ID No. 119: C-L-R-A-P-R-S-P-K-M-V-Q
SEQ ID No. 120: L-R-A-P-R-S-P-K-M-V-Q-C
SEQ ID No. 121: C-L-R-A-P-R-S-P-K-M-V-Q-G-S
SEQ ID No. 122: C-L-R-A-P-R-S-P-K-M-V-Q-G-S-G.

23 (currently amended): A method for obtaining anti-proBNP(1-108) antibodies that specifically recognize the sequence $Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{85}$, (SEQ ID No. 4) the sequence $Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{84}$ (SEQ ID No. 108) and/or the sequence $RAPR_{76}S_{77}P$ (SEQ ID No. 5) of proBNP(1-108) with the substantial exclusion of the BNP(1-76) and BNP(77-108) peptides, and that have the ability to specifically recognize circulating proBNP(1-108) in human serum or plasma samples, in which method an animal is immunized with a peptide as defined in claim 16, and, optionally, the antiserum obtained is depleted using the BNP(77-108) peptide and/or the BNP(1-76) peptide.

24 (currently amended): A method for obtaining a hybridoma that secretes an anti-proBNP(1-108) antibody that specifically recognizes the sequence sequence $Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{85}$ (SEQ ID No. 4), the sequence $Y_{70}TLRAPR_{76}S_{77}PKMVQGS_{84}$ (SEQ ID No. 108) and/or the sequence $RAPR_{76}S_{77}P$ (SEQ ID No. 5) of proBNP(1-108) with the substantial exclusion of the BNP(1-76) and BNP(77-108) peptides, and that have the ability to specifically recognize circulating proBNP(1-108) in human serum or plasma samples, in which method an animal is immunized with a peptide as defined in claim 16, immunoglobulin-secreting lymphocytes are removed from this animal, and the lymphocytes are fused with myeloma cells so as to obtain at least one immunoglobulin-secreting hybridoma.

25 (original): An anti-proBNP(1-108) antibody, characterized in that it is obtained by a method as claimed in claim 23.

26 (currently amended): The anti-proBNP(1-108) antibody as claimed in claim 25, which specifically recognizes the sequence Y₇₀TLRAPR₇₆S₇₇PKMVQSGS₈₅ (SEQ ID No. 4) or the sequence Y₇₀TLRAPR₇₆S₇₇PKMVQGS₈₄ (SEQ ID No. 108) of proBNP(1-108).

27 (original): A hybridoma which can be produced by the method as claimed in claim 24.

28 (original): An anti-proBNP(1-108) monoclonal antibody secreted by a hybridoma as claimed in claim 27.

29 (original): The anti-proBNP(1-108) monoclonal antibody as claimed in claim 28, secreted by the hybridoma 3D4 deposited with the CNCM under the No. CNCM I-3073.

30 (currently amended): A method of in vitro diagnosis of heart failure in a human subject, comprising bringing a biological sample of the human subject into contact with an anti-proBNP (1-108) antibody as defined in claim 25[,] and detecting the proBNP (1-108) in the sample, whereby, if the proBMP (1-108) concentration in the biological sample is higher than that of normal individuals, then the human subject is diagnosed as having heart failure.

31 (currently amended): A method of *in vitro* diagnosis of heart failure in a human subject, comprising:

- a) bringing a biological sample of the human subject into contact with an anti-proBNP(1-108) antibody as defined in claim 25,
- b) incubating the mixture under conditions that allow the formation of antigen-antibody complexes, and
- c) revealing the antigen-antibody complexes formed, optionally using a labeled detection antibody capable of binding specifically to the proBNP(1-108) present in the primary complex, or using a labeled detection antigen capable of binding to the antibody directed against said proBNP(1-108) present in the primary complex, and
d) correlating the amount of antigen-antibody complexes revealed with the clinical condition of the human subject, wherein if the amount of antigen-antibody complexes is higher than that of normal individuals then the human subject is diagnosed as having heart failure.

32 (cancelled).

33 (previously presented): A kit for detecting proBNP(1-108) in a biological sample, containing at least one antibody as defined in claim 25.

34 (previously presented): A kit for detecting proBNP(1-108) in a biological sample, containing, as standard and/or control, at least one peptide as defined in claim 16.

35 (currently amended): A kit for detecting proBNP(1-108) in a biological sample, containing:

- in a container, at least one antibody as defined in claim 25;
- in another container, at least one peptide of formula:



where

a_1 may be H or may represent a function or a chemical group chosen from a thiol, alcohol, aminoxy, primary amine or secondary amine function, an aminocarboxyl group, a biotinyl group or an acetyl group,

X_1 represents a peptide sequence of 0 to 3 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

X_2 represents a peptide sequence of 0 to 7 amino acids, which may or may not be derived from the natural sequence of proBNP(1-108),

a_2 may represent an OH function, an NH_2 function, or an alkoxy group.